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A CONTRIBUTION TO THE DEVELOPMENT OF PARYPHA CROCEA.¹

CARRIE M. ALLEN.

My work has been carried on at the Zoölogical Laboratory of Syracuse University under the direction of Dr. C. W. Hargitt, to whom it is a pleasure to express my obligations for his kind suggestions and supervision throughout its progress, and for the pains which he has taken in furnishing me with the best of appliances and material. The material upon which the investigations have been made was collected by Dr. Hargitt at Woods Holl, Mass., during the summer of 1898. Through his kindness I had at my disposal an almost limitless supply killed and preserved by a number of methods. Picro-sulphuric acid and Perenyi's fluid both gave excellent results, but formalin proved unsatisfactory for histological work.

In most of my study I used preparations stained *in toto* in borax-carmin. The specimens were left in alcoholic borax-carmin for twelve hours, after which the stain was extracted by acid alcohol for from fifteen minutes to half an hour. In dehydrating they were left in each of the various grades of alcohol for thirty minutes, after which they were cleared either in cedar oil or chloroform. Both clearing agents gave good results, but the latter was preferable because of its rapid action.

In staining on the slide, iron-haematoxylin and double stain of eosin and haematoxylin both proved satisfactory.

In using the iron-haematoxylin the sections were fixed to the slide, carried down through the alcohols to 50 per cent, and placed in a 2 per cent solution of ammonio-ferric-alum for from thirty minutes to three hours. They were then washed in running water for twenty minutes, stained in 0.5 per cent aqueous solution of haematoxylin for from one-half hour to

¹ Contributions from the Zoölogical Laboratory, Syracuse University.

two hours, washed again in running water and cleared for a few seconds in iron-alum. After rinsing in distilled water they were plunged into 95 per cent alcohol, carried up and mounted in balsam. The best results were obtained by leaving the slides in each stain for an hour. This method was of greatest value in study of the segmentation of the egg. The cytoplasm appeared gray, chromatin fibers black.

In using the eosin-haematoxylin method the sections were stained for an hour in a 2 per cent solution of eosin in 90 per cent of alcohol, after which they were stained in a weak solution of Delafield's haematoxylin for twenty minutes. This stain gave very good general differentiation, and was of especial value in determining the origin of the sex cells.

A number of other stains and combinations of stains were used with fair success.

General Description of Parypha.

Parypha crocea is found all along the New England coast attached to floating timbers and the piles of wharves. It seems to prefer brackish water and partial sunlight, but often occurs in pure sea water.

This hydroid grows in colonies which arise from a single individual by a process of budding, and the sexes are always separate. The hydrorhiza is made up of a contorted mass of irregularly branched stems, from which the hydrocaulus of the individual hydroid arises. The stems bearing the adult polyps are usually two and a half to three inches in length, and short stems are sometimes found branching out from the main ones. Enclosing the stem is a horny, often annulated sheath, the perisarc. The polyp is borne at the top of a somewhat globular expansion of the hydrocaulus, and is almost conical in form, with a broad, saucer-shaped base. It contracts about three-quarters of the way up, forming a thick-walled, flexible proboscis, in the center of which lies the mouth, surrounded by a circle of short, thick tentacles with decurrent bases. Around the base of the polyp is a circle of long, slender tentacles, varying in number from sixteen to twenty-four. The medusoids

are borne upon long, slender, branched peduncles which arise a short distance above the tentacles of the lower row. All parts of the hydroid are made up of the two layers characteristic of all hydroids, but the mesogloea forms only a thin layer in the peduncles and tentacles and is not visible in the medusoids. The tentacles of both rows consist of a central axis of the endoderm, surrounded by a thin layer of ectoderm.

Origin and Morphology of Male Gonophores.

The medusoids in this species begin to appear early in the development of the hydroid, when the head upon which they are borne is less than a quarter of the size of the adult polyp. The first indication of their formation is a slight outpushing of the endoderm of the body wall a little above the axils of the lower row of tentacles. The ectoderm is pushed out and becomes thinner than in the adjacent parts of the wall. The papilla thus formed elongates into a peduncle communicating directly with the body-cavity of the polyp. From this peduncle arise short branches which may subdivide, and it is at the ends of these that the medusoids are borne. At first there is merely a thickened layer of endoderm surrounded by a thin layer of ectoderm, but when the length of the bud is about once and a half the width, the ectoderm cells at the tip begin to grow rapidly, forming a plug of cells with large nuclei and indistinct boundaries (Pl. I, Fig. 1). For a time the endoderm is forced back (Pl. I, Fig. 2), but it soon begins to grow down into the center of the plug, to form the manubrium, and around the outside to form the endodermal layer of the bell. All the cells between this layer and the manubrium are of ectodermal origin, and from them the reproductive elements arise. The sex cells increase in number, and to some extent in size, until they occupy the greater part of the bell. While this growth is taking place the cells at the distal end of the gonophore next the endodermal layer of the bell begin to differentiate, forming a thin, delicate layer which gradually extends around the gonophore and becomes the inner wall of the bell (Pl. I, Fig. 3). It is made up of cells much smaller than those from which they

are derived. Meanwhile the endodermal layer has thickened in four regions equidistant from each other at the distal end of the medusoid. At first the cells in the thickened region are irregularly arranged, but later they form themselves into two rows with a space between them. In a few cases no cavity was found, and in two of the gonophores examined it extended halfway around the bell. The remainder of the wall consisted of a single layer. Pl. I, Fig. 4, represents the condition found in most of the medusoids. Agassiz ('62), p. 259, states that there are neither radial nor circumoral canals in this species but the position and mode of development of these cavities leave little doubt that they are rudimentary radial canals. No circular canals were observed, and the radial canals were never found connected with the body cavity of the medusoid in any of the hundreds of sections studied.

Spermatogenesis.

The large nucleated cells lying between the manubrium and the inner wall of the bell become the sperm mother-cells, which finally break up to form the sperms. In the first division the karyokinetic figures are distinct and show spindles and prominent chromosomes. The later stages were difficult to study because of the minuteness of parts, and I was unable to demonstrate clearly the exact number of spermatozoa derived from a single germ cell, but I think four are usually formed. Their structure could only be made out in particularly favorable sections, but was easily demonstrated by crushing the gonophore and allowing the sperms to escape. They consist of a pear-shaped head with a very long, slender tailpiece. When fully developed the male gonophores are spherical, and the walls are so thin that their structure can only be determined by the use of very high powers. They bear no tentacles, although the ectoderm is sometimes thickened slightly in the regions where tentacles arise in the female. I examined carefully a large number of mature male gonophores to learn whether or not the ectodermal layer of the manubrium was formed and discovered a definite transparent layer next the

endoderm. No gonophores from which the sperms had been expelled were found, so I was unable to prove that this represented the ectoderm of the manubrium, but as that layer was not observed in the female until a very late stage, I think that there can be little doubt that it functions as such.

Origin and Morphology of the Female Gonophore.

The female gonophores arise in the same manner as the male and, in the early stages, are made up of the same parts, but later may always be distinguished by a circle of six or eight short, blunt tentacles at the distal end and by their more elongated shape. When filled with young they are nearly spherical and the tentacles mere papillae; but when the larvae have been set free the medusoids become elongate and the tentacles expand. Pl. I, Fig. 5, shows a section through two of these tentacles.

Oögenesis.

The primitive egg cells are developed in the same manner as the sperm mother-cells. I find no evidence whatever of ova either in the coenosarc of the stem, the body of the polyp, or the walls of the peduncle. There are in the endoderm of the polyp and peduncle numerous large, deeply stained cells with large nuclei which somewhat resemble eggs when cut in the right plane, but a careful study of a large number of sections reveals the fact that they are in reality highly differentiated endodermal cells. They are always in contact with the supporting layer and usually project beyond the other endoderm cells into the body cavity, neither of which conditions, according to Weismann ('83), p. 70, occurs in egg cells. Moreover, these cells are much larger than the primitive ova and take a deeper stain than do the eggs in any stage of their development. They are very rich in protoplasm, and sometimes the outer surface is found sloughing off into the body cavity. This condition was even more marked in similar cells in *Eudendrium ramosum*, where they extend farther into the body cavity, and the discharge of portions of their protoplasm was

very evident. All this would indicate that they were glandular in function. They are largest and most numerous in the peduncle and occasionally one is found in the manubrium, but such cases are rare. It would be impossible to distinguish a section through the peduncle of a male head from that of a female, as these cells are equally conspicuous in both. Pl. I, Fig. 8, shows a number of these glands, one of which resembles an egg, but other sections through the same peduncle show that it is really in contact with the supporting lamella.

In the younger stages of development the manubrium of the female appears to consist entirely of endoderm, but when the gonophore is fully mature and the primitive ova have disappeared, a thin layer of ectoderm is found to be present. It consists of a single layer of much flattened cells with smaller nuclei than those of the germ-tissue cells from which they are derived.

Development of the Ovum.

The primitive egg cells make up the large mass of tissue lying between the manubrium and the inner wall of the bell. They are packed closely together, so that the outlines of the cells are more or less irregular. The nuclei are large and spherical and contain a prominent nucleolus which takes a very deep stain. The mass of protoplasm surrounding each nucleus is small, and the cell boundaries are very indistinct (Pl. I, Fig. 4).

As the gonophore grows older the nuclei of the germinal tissue become much larger and more prominent, the mass of protoplasm surrounding them increases in bulk, and the cell boundaries become more clearly defined. At this stage the nuclei appear as very large spheres, with the chromatin fibers arranged in a sort of network around the periphery. Within the layer of chromatin is a colorless mass, near the center of which lies the nucleus suspended by four or five slender threads, which run out to the layer of chromatin. These threads take a fainter stain than the chromatin fibers and are only visible in especially well prepared specimens.

The nucleolus is usually spherical or slightly elongated,

but in many cases it shows a varying number of short, blunt processes. This condition was most clearly seen in sections stained with ammonio-ferric-alum and haematoxylin.

Within the nucleolus are a number of small, transparent, highly refractile bodies, the nature of which will be discussed later. There is usually one of these in the nucleus of each primitive ovum, but some contain two. As the ova grow older the number increases and there are sometimes as many as four or five in a single nucleolus. The protoplasm of the cells is granular and often contains a few small vacuoles.

Up to this time the growth of the various cells of the germinal tissue has been about equal, but now several cells increase markedly in size, and often the greater number in one side of the gonophore are found to be thus growing. If, however, a large number take part in this early development, the cells in the opposite side of the gonophore decrease in size, both nucleus and cytoplasm becoming smaller. Soon a few cells attain greater size than the rest and develop very rapidly. Many of the cells in this and the preceding stages are found to possess pseudopodia-like processes quite similar to those figured by Doflein ('96) for Tubularia. Smallwood ('99) mentions the same condition in the eggs of Pennaria. The pseudopodia extend in between the other primitive egg cells, and the tips are more granular and take a deeper stain than the rest of the egg. Doflein ('96) has given much attention to the amoeboid forms assumed by eggs of Tubularia, and he inclines to the belief that these processes do not function as mouths by which the surrounding eggs are bodily engulfed. My results have coincided very closely, in most respects, with those of Doflein, but numerous cases were also observed where the outline of the absorbed egg could be definitely made out within the protoplasm of the absorbing egg. Even in these cases, however, the absorbed egg did not lie in a vacuole, as would the food taken in by the amoeba, and the outline could only be made out by the greater density of its protoplasm. It seems, therefore, that in this case also we have a blending of the protoplasm of the two cells rather than a digestion and absorption of the one by the other. There seems to be no great uniformity either

in the number or location of the primitive cells which finally become ova, although by far the greater number lie next the manubrium, and few, if any, develop on the outer surface of the germinal mass. I am inclined to agree with Doflein ('96), p. 65, that all of the cells of the germinal tissue have potentially the capacity of becoming eggs, but that those favored by better nourishment or advantage of position are the first to develop.

He says in this connection : "Das starke Wachstum des Gonophors hat einzelne Lücken und Spalten im Gewebe entstehen lassen, und in diese wachsen nun die Keimgewebezellen mit ihren Fortsätzen hinein." But while in my investigations many such cracks were found, in most instances the pseudopodia extended between eggs where no crack occurred, and in the greater number of ova no pseudopodia were present at all. I am, therefore, led to the belief that proximity to cracks in the germinal tissue is not of controlling importance, although the eggs do undoubtedly take advantage of the room afforded by such cracks when present. Doflein ('96) also states that in *Tubularia* the growing eggs are always found next the manubrium or upon the outside of the germinal mass, unless cracks are present within the tissue. I have examined a large number of sections, and I find that in *Parypha* the eggs of the outer layer are the last to develop, but that those in the interior of the germinal tissue are often found considerably enlarged even in the younger gonophores.

When the growing cells have attained a diameter about three or four times that of the cells of the germinal tissue, the nucleus is found lying close to the periphery of the egg and is oval and transparent, the chromatin fibers being scarcely visible (Pl. II, Figs. 5, 6). The nucleolus takes a fainter stain, and in most cases contains a number of the refractile bodies already mentioned. Later these bodies apparently unite, as nearly the whole nucleus is often occupied by a single large one. Just what their character is I am unable to state, but they appear to contain oil, and certainly they are associated with the peculiar metabolism exhibited by the cell at this time (Pl. II, Figs. 5-7). In some of the eggs in which the nucleus had this peripheral position, its outline was irregular upon the

inner side so that it resembled the figure shown by Hickson ('90) to illustrate the stage in the fragmentation of the oö sperm nucleus of *Allopora*. In the next stage the nuclear membrane is broken down and the nucleoplasm blends with the cytoplasm of the egg, from which it can only be distinguished by its homogeneousness and greater transparency (Pl. II, Figs. 7, 8). In other eggs having the same general appearance as the last no nucleus whatever is visible. I have several complete series through eggs in this stage, none of which show any signs of a nucleus, although they have been stained by a number of different processes, and I am perfectly confident that the nucleus would be visible if present. Hickson describes a similar condition in the eggs of *Allopora*, *Milleopora*, and *Distichopora*; and Dr. C. W. Hargitt tells me that in his opinion a like condition is to be found in *Eudendrium*, although he has not yet placed it beyond doubt.

Hickson ('93) has written an extended account of "nuclear fragmentation," in which he cites the opinion of a number of authors with regard to this much disputed question. After describing the stages observed in *Distichopora* he says: "I have described a process which can only be compared with the so-called free nuclear formation in the early insect embryos. Nuclei make their appearance in places which were previously devoid of any nucleus or nuclear structure. It is not reasonable, however, to assume on the insufficient evidence before us that "nuclear formation" does actually occur. It seems to me much more probable that minute fragments of nuclear substance scattered through the protoplasmic meshwork collect together in places, and form by their fusion true recognizable nuclei. In other words, the process we have under observation is rather one of "nuclear regeneration" than one of free "nuclear formation." He quotes Flemming and Ziegler as authorities most opposed to this view, both these investigators contending that any process of nuclear division other than that by mitosis is a sign of the degeneration of the nucleus and the approaching end of the life of the cell. Ziegler inclines to the opinion that nuclei which have arisen by amitotic division will never again divide mitotically. Opposed to these are the works

of Verson, Frenzel, Löwit, and others who, since the publication of Ziegler's paper, have called attention to cases of amitotic division of the nucleus which are certainly not followed either by nuclear degeneration or a cessation of cell multiplication. Altogether there seems to be constantly increasing evidence that such a fragmentation does occur in the ova of widely separate groups of animals.

Wilson ('96), p. 85, believes that the subject requires more study, but says: "There can be no doubt, however, that Flemming's hypothesis in a general way represents the truth, and that in the majority of cases amitosis is a secondary process which does not fall in the generative series of cell division."

Absorption.

At about the time when the transparent nucleus lies near the periphery of the egg the cytoplasm changes from a granular to a reticular structure. The boundaries between the large cells and those adjacent to them now begin to break down and the protoplasm to blend. This fusion may take place between two large cells or between a growing cell and a germ tissue cell. The former usually occurs first, the large cells near the manubrium fusing and then gradually taking in the germ cells which surround them. The nuclei of the latter are found lying in the protoplasm of the absorbing cell. Both conditions are shown in Pl. I, Figs. 6, 7.

The outline of the syncytium thus formed is very irregular, and parts of the walls of the constituent cells persist for a time, showing where the fusion has occurred (Fig. 7). Doflein ('96), p. 66, states that in *Tubularia* one large, well-nourished cell controls the absorption, and that as it grows its nucleus also increases in volume, and that the nucleus becomes the functional nucleus of the ovum, the other nuclei being gradually absorbed. In *Parypha*, as already stated, the nuclei of the growing cells disappear at an early stage so that only the nuclei of the smaller cells persist. It thus becomes impossible to tell which is the controlling cell.

Finally the mass of fused cells takes on the typical egg

form, the protoplasm near the periphery becomes more dense, and the absorbed nuclei are found in various stages of disintegration. The egg now lies on the outside of the mass of germinal tissue and next to the wall of the bell. No evidence of fusion with the primitive eggs was observed after this stage was reached, although the two were still in contact. It is quite evident, however, that the remaining germ cells grow and unite to form new eggs later in the history of the parent, since primitive eggs are often found in advanced stages of growth, while two or three nearly mature embryos still occupy the gonophore. In other cases the gonophores contained several embryos in various stages of development, but no primitive ova. Doflein ('96), p. 67, states that, although he was unable to obtain sections to illustrate adequately the point, he believes that the germ cells of *Tubularia* do unite to form new eggs after the larvae have left the gonophore. In *Parypha* there is no chance to doubt that new ova are formed even before the exit of the larvae.

Fertilization.

As is the case in many of the hydroids, the process of fertilization is shrouded in mystery. The fact that the eggs are developed in closed gonophores makes it difficult to decide just when fertilization takes place. In discussing the development of *Allopora*, Hickson states that he believes that fertilization occurs while the nucleus lies at the periphery of the egg, and previous to the time when it becomes irregular in outline. From the positions of the eggs in which these irregular nuclei were found, *i.e.*, next the manubrium, this might be the case here, but nothing was discovered which threw any light directly upon the matter.

History of the Pseudo-Cells.

The nuclei of the absorbed cells are found in various stages of disintegration within the ovum. Some of them resemble so closely the nuclei of the germ-tissue cells that, were it not for the position and the vacuoles within which they lie, it would be

impossible to distinguish them. Later the chromatin fibers lose their reticular arrangement and assemble into a varying number of small spheres just within the periphery of the nucleus, and at the same time the threads which support the nucleolus disappear. The ground material in which the chromatin is suspended, and which up to this time has been nearly transparent, now begins to react to the staining agents, and the structure of the nuclei becomes obscure. If, however, methyl-blue was used, this substance was only slightly affected, so that this stain proved most satisfactory for the study of the various phases exhibited by the retrograding nuclei, or pseudo-cells, as they are sometimes called. Many of the nuclei are often found in the process of division. The nucleolus lengthens slightly, and finally separates into two parts. Later the entire nucleus divides and part of the chromatin goes with each half. Cases in which the nucleolus had divided were very numerous, but very few were found in which the division was actually taking place. Pl. II, Fig. 11, shows such a one, and Fig. 10 represents a nucleus in which there were three processes on the nucleolus. No chromatin fibers were visible in either of these cases. The halves thus formed often divided again, sometimes before they were separated, and in some instances as many as six parts can be observed. The chromatin globules vary in number and size in the various parts (Fig. 12). In some of the nuclei the division is less regular, and portions are often found in the process of being absorbed into the protoplasm of the egg. Fig. 15 represents a nucleus in which the parts formed by the first division were of very unequal size. In the smaller the nucleolus has again divided, but the larger part has been partially absorbed. Often several of these nuclei are found in a single vacuole. Fig. 9 shows one in which there were seven in various stages of disintegration, but usually not so many are found. Doflein believes that they are carried into the vacuoles by currents in the protoplasm. All this goes to strengthen the opinion of Doflein that the absorbed nuclei take the place of the yolk-granules, which are wanting in this species, and that they are gradually broken down to serve as food for the developing egg. They persist through the entire

embryological development, being very numerous in the endoderm of the young hydroid when it escapes from the gonophore. Isolated ones are even found in the endoderm of the tentacles, as noted by Doflein, but I cannot agree with him that they are entirely confined to that layer.

Segmentation of the Ovum.

The egg, after assuming the typical form already described, goes into a resting stage, as a large number are found in that condition and without nuclei. Soon, however, an irregular mass of nuclear matter appears at one pole. Sometimes this forms a single mass, in other cases it is made up of two or three more or less isolated portions. Whether these are finally assembled to form a single nucleus, or whether two or three nuclei are thus produced, I am unable to say, as many of the sections in the later stages might be interpreted either way. In some of the eggs a single definite star-shaped nucleus was present, but in others there were two, and in one case four of these nuclei lying close together at one pole of the egg. There was nothing in these eggs to indicate that the nuclei had not been derived from a single nucleus, but, on the other hand, some of the disorganized masses of nucleoplasm could not but give the impression that more than one would be formed. However, the number is of minor importance, and the real interest attaches to the fact that such a reorganization occurs at all. That it does, I am fully convinced. I have examined a large number of sections with this question particularly in my mind, and am forced to the conclusion that the nucleus of the mature egg is formed by the reorganization of the fragments of the nuclear matter scattered through the cytoplasm.

The earliest stage in which definite mitosis was observed was in the egg shown in Pl. III, Fig. 1. In this three definite nuclei, one in a process of division, showed in a single section. Another section through the same egg revealed a fourth nucleus which, from its position, might have been derived from one of the others, but no spindles were observed. There were no signs whatever of segmentation planes in this egg. The development in the eggs of *Parypha* is very irregular indeed,

and seems to be governed by no single law. In some cases definite cell walls were found in the earlier stages, as in Fig. 2, where four cells had been formed, one of which contained two nuclei. In this we have only the stage next to the one last described, but in that there were no segmentation planes at all. In still later stages the development is quite as irregular. Fig. 5 shows a section in which six nuclei were visible, and other sections through the same egg contained several others, some of which were in the process of division, but no cell walls had been formed. In Figs. 6 and 7 we have sections through much older eggs, but the same indefiniteness of structure prevails. From the foregoing illustrations it will be seen at once that there is little uniformity in the early development of the eggs of *Parypha*, either as to size of the cells formed or the number of nuclei that appear previous to the formation of the cell walls. Segmentation does, however, begin at one pole, and the greater part of the egg is for a time unsegmented. In no case did I find the egg divided into two equal parts, as Dr. Hargitt has sometimes observed in *Pennaria* eggs. Pl. III, Fig. 3, represents conditions similar to what is constantly met with in eggs of *Pennaria*. In total segmentation the ovum consists of a solid sphere of cells of more or less uniform size but with irregular outlines. They are very reticular in structure, and large vacuoles are numerous.

Formation of the Ectoderm.

Following the complete segmentation, the first indication of a differentiation into ectoderm was observed in an increased amount of cytoplasm in the outer layer of cells. These cells then divide radially, forming narrow cells, as shown in text Fig. 1. The two mitotic figures lay in adjacent cells, as shown in the drawing. In the next stage observed the ectoderm appeared to consist of two layers of cells much smaller than those of the endoderm, and distinguished from them by the greater density of protoplasm. The two layers appeared in this case to be dovetailed into each other, as shown in Fig. 2 of the text. In the fully formed ectoderm the cells are very elongate and somewhat

spindle-shaped, with one end broader than the other. Both the form of the cells and the position of the nuclei indicate that they have been formed from a condition like that in Fig. 2, and not by further delamination of the outside layer alone. At this stage the larva is made up of a solid mass of irregular cells with spherical nuclei surrounded by a single layer of much elongated cells. No segmentation cavity is formed. The origin of the germinal layers agrees, therefore, quite closely with that described by Hickson ('93) under *E. e.*, p. 52: "A sterula is formed by precocious delamination. No segmentation cavity is formed, and segmentation is at first incomplete." Parypha is not mentioned by Hickson under this class, and

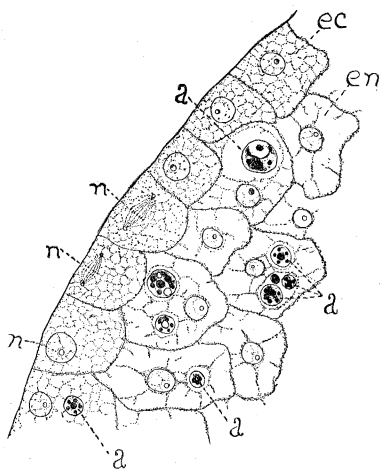


FIG. 1 \times 560. — Early stage in the formation of the ectoderm of the embryo; *ec*, ectoderm; *en*, endoderm; *n*, nuclei; *n*¹, nuclei in the process of division.

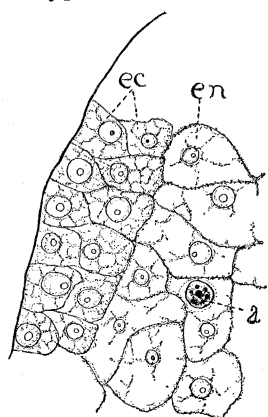


FIG. 2 \times 560. — Later stage in the formation of the ectoderm; *ec*, ectoderm; *en*, endoderm; *a*, nuclei of absorbed cells.

Tubularia, the form most like Parypha in its general mode of development, he includes under another head. Dr. Hargitt informs me that nothing equivalent to true delamination or invagination occurs in Pennaria. It would, therefore, seem that no one, two, or even three laws of cleavage are sufficient to explain the varied conditions to be found in the segmentation of the hydroid egg.

The embryo now appears concave upon the side next the manubrium, but this is probably due to pressure and not to any intrinsic cause. After the formation of the ectoderm the two layers of the embryo evaginate at seven points so that a section through the region of the process appears star-shaped,

the rays at first being very short. These processes elongate to form the basal tentacles of the young hydroid. While this growth is taking place the convex side of the embryo becomes still more convex, and the concave portion between the tentacles evaginates and becomes convex also. In this way the endoderm cells in the center are split apart and the body cavity is formed. At first it is very irregular, but later the endoderm cells assume the typical endodermal form and arrange themselves in a single layer within the ectoderm, and the body cavity

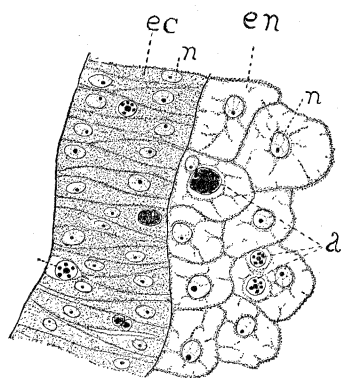


FIG. 3.

FIG. 3 $\times 560$. — Fully formed ectoderm from convex side of embryo; *ec*, ectoderm; *en*, endoderm; *n*, nuclei; *a*, nuclei of absorbed eggs.

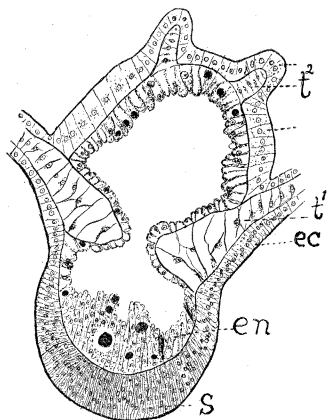


FIG. 4.

FIG. 4 $\times 190$. — Young embryo ready to escape; *t*¹, basal tentacle; *t*², buccal tentacle; *s*, stalk; *ec*, ectoderm; *en*, endoderm.

takes on a form quite similar to that of a young polyp. From almost the earliest stage in the development of the ectoderm the cells on the convex side of the embryo appear much longer than upon the opposite side, and it is this portion which becomes the stem to which the young hydroid attaches itself. According to Agassiz the larva escapes in this condition, and the mouth and buccal tentacles are developed after it attaches itself. I have, however, obtained sections of a large number of mature larvae in which well-developed tentacles were present. Fig. 4 represents an embryo that was just leaving the gonophore. The body and the stem were both well developed, and the basal tentacles were nearly twice as long as the body.

At the buccal end there were five or six tentacles, a section of which is shown in the figure. Whether the mouth is developed at this time or later I did not decide.

Summary and Conclusion.

In a summary of the results obtained in this study, the following points should be noted:

1. The medusoid develops from a bud formed by an outgrowth of the body wall and shows itself first in a thickening of the endoderm.

2. The sex cells in both the male and the female are derived from the plug of ectodermal cells which is formed at the apex of the bud.

3. The medusoid is never set free and no circular canal is formed, although remnants of four radial canals are quite conspicuous.

4. The eggs grow by the absorption of the cells of the germinal tissue, a syncytium being thus formed.

5. The nuclei of the primitive eggs persist as pseudo-cells and are gradually broken down to serve as food for the growing embryos.

6. The pseudo-cells divide amitotically, but are finally absorbed by the growing egg.

7. The nucleus of the growing egg is absorbed at an early stage, but is re-formed, after the assumption of the typical egg form, from the fragments scattered through the protoplasm.

8. Segmentation is very irregular and nuclear division often outruns the segmentation of the egg.

9. The ectoderm is formed by radial delamination of the two outer layers of cells.

10. The embryo escapes as an actinula with both basal and buccal tentacles.

The results obtained in this investigation differ in several points from those of Agassiz, whose description of *Parypha crocea* is the only one that I have found. Clark ('93), to be sure, refers to the eggs and spermatozoa of this species, but gives no account of them. Agassiz states that he was unable to

find any trace of eggs, and that the embryos are developed from a large spherical portion which buds off from a granular mass of protoplasm formed by the separation of the endoderm and ectoderm in the medusoid bud. This granular mass he calls the "germ basis." A study of stained specimens in section shows clearly that this granular mass, or "germ basis," as he calls it, is really the mass of sex cells which have already been described. His opinion that the embryo was formed by the budding off of large portions of this mass probably arose from the fact that in the early stages of the development the eggs are packed closely together and the membranes are indistinct, so that the whole mass appears somewhat homogeneous. As the eggs grow, they become less granular and in time are entirely separated from the germ tissue. As to the radial canals, they would probably be overlooked, except in sections, as they are never functional. The tentacles of the embryo are, however, so well developed that it seems strange that he should not have observed them, since he has noted tentacles upon the female gonophore where they are less clearly defined.

SYRACUSE UNIVERSITY, May, 1900.

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EXPLANATION OF PLATE I.

FIGS. 1-7 $\times 190$; FIG. 8 $\times 270$.

FIG. 1. Young medusa bud showing the formation of the germinal cells from ectoderm of bud. *ec*, ectoderm; *en*, endoderm; *h*, germinal cells.

FIG. 2. Later stage, germinal cells separated from the ectoderm of the bud.

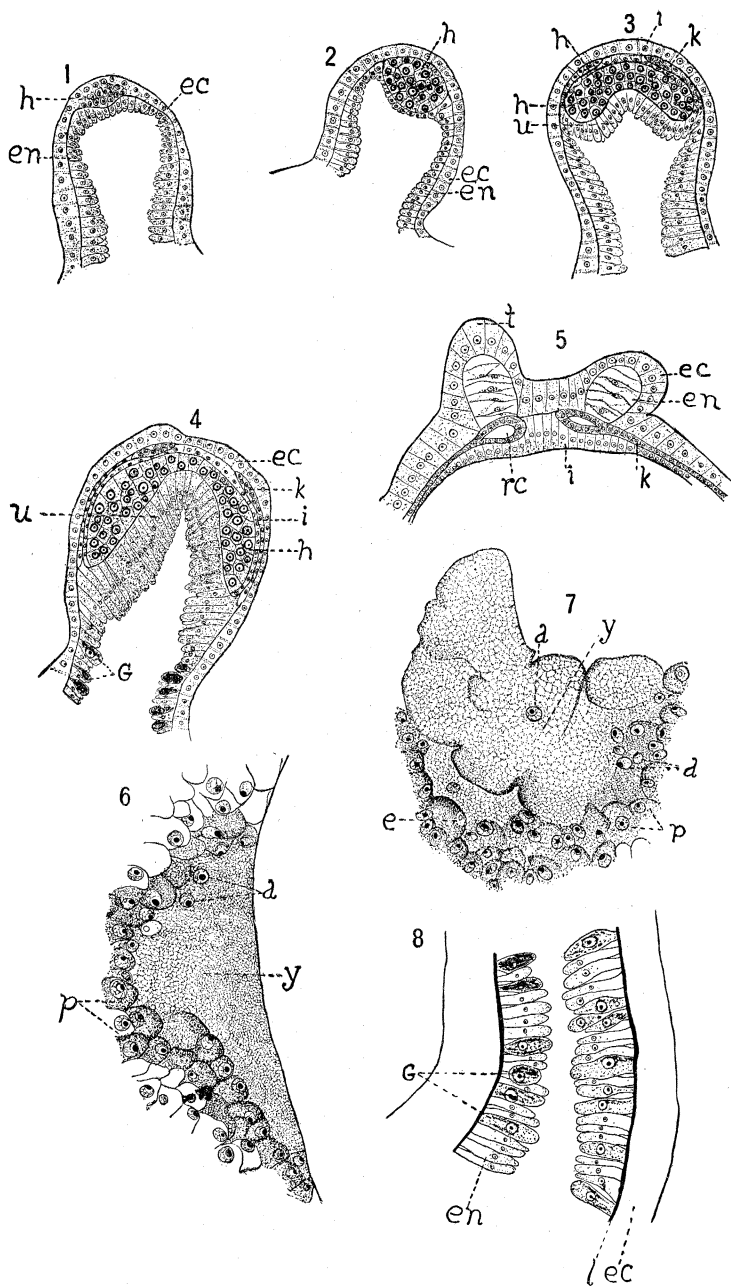
FIG. 3. Still later stage showing the mode of formation of the endodermal layer of the bell (*k*), the inner ectodermal layer (*i*), and the manubrium (*u*).

FIG. 4. Showing the layers of the bell completely formed.

FIG. 5. Distal end of mature female gonophore showing tentacles (*t*), rudimentary radial canals (*rc*), outer ectodermal layer of the bell (*ec*), and endodermal layer (*k*), inner ectodermal layer (*i*).

FIGS. 6, 7. Showing growth of the egg by the absorption of the primitive egg cells (*p*). Syncytium thus formed (*y*); nuclei of absorbed cells (*a*); young growing cell (*e*).

FIG. 8. Longitudinal section through peduncle from female head showing gland cells (*G*).



EXPLANATION OF PLATE II.

FIG. 1 $\times 127$; FIG. 2 $\times 118$; FIGS. 3-8, 10-15 $\times 765$; FIG. 9 $\times 495$.

FIG. 1. Growth of ovum (*z*) by absorption of primitive cells (*p*).

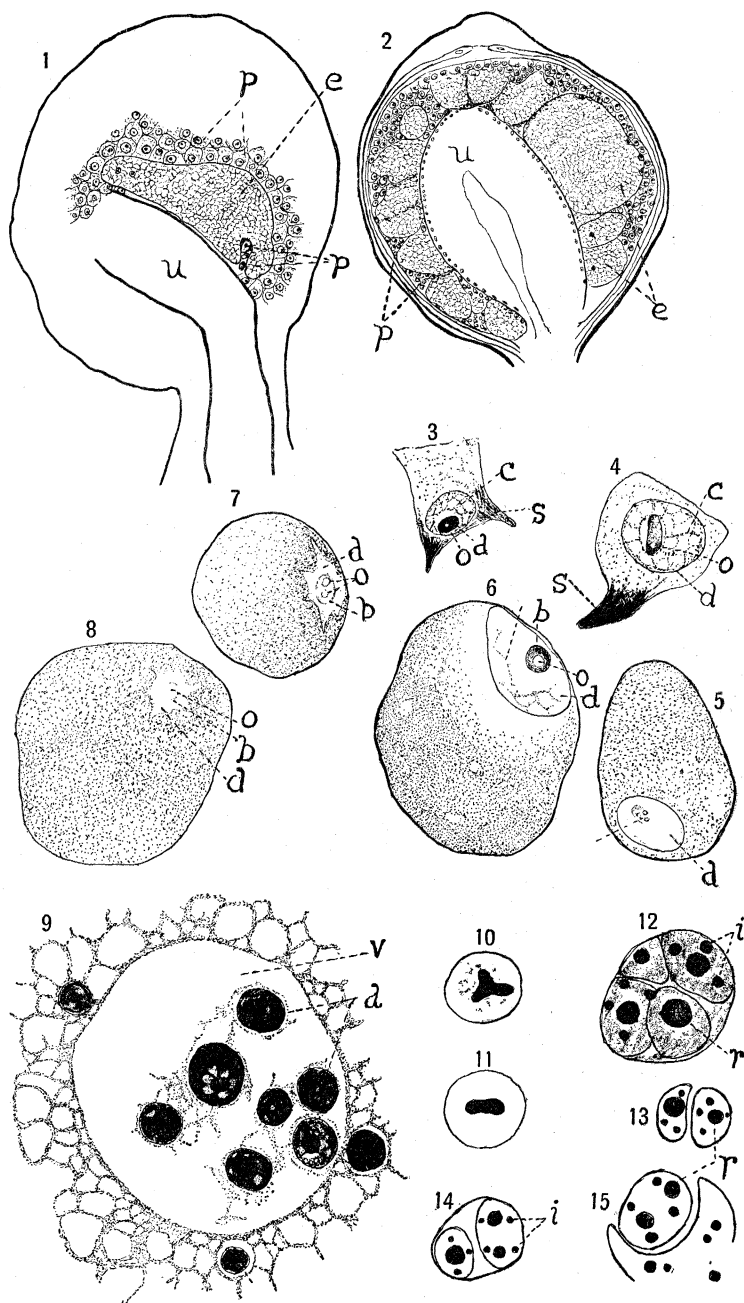
FIG. 2. Showing the number of growing primitive egg cells to be found in a single gonophore.

FIGS. 3, 4. Primitive egg cells in early stage of development showing pseudopodia (*s*); nucleus (*d*); oil drops (*o*); chromatin fibers (*c*).

FIGS. 5-8. Later stages showing the disappearance of the nucleus of the growing egg. *d*, nucleus; *b*, nucleolus; *o*, oil drops.

FIG. 9. Vacuole (*v*) in segmenting egg showing nuclei of absorbed cells (*a*) in various stages of disintegration.

FIGS. 10-15. Retrograding nuclei of absorbed cells. Nucleolus (*r*); assembled chromatin fibers (*i*). In Fig. 15 a portion of the nucleus has been absorbed; the smaller part is in the process of division, the nucleolus having already divided.



EXPLANATION OF PLATE III.

FIGS. 2, 6 \times 115; FIGS. 1, 3-5, 7 \times 152.

FIG. 1. Young ovum showing three nuclei (*n*). *n*¹ in the process of division. No segmentation planes visible; *a*, nuclei of absorbed cells.

FIGS. 2, 3. Slightly later stages; segmentation planes well marked. *n*, nuclei; *a*, nuclei of absorbed cells.

FIG. 4. Still later stage of segmentation.

FIG. 5. Section through an egg containing fifteen or sixteen nuclei, but no well-defined cell walls. *n*, nuclei; *a*, nuclei of absorbed cells.

FIG. 6. Later stage; cell boundaries indefinite.

FIG. 7. Advanced stage of segmentation; cells irregular in outline, cytoplasm very reticular; *n*, nucleus; *n*¹, nuclei in process of division; *a*, nuclei of absorbed cells.

